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2

July 12, 1991

A.J. Melaragno
Captain, Medical Corps
United States Navy
Director of Research and Development
National Naval Medical Center
Bethesda, Maryland 20814-5044

RE: Status Report for Grant # N00014-90-J-1847
Entitled "Development of Hematopoietic Growth Factors for Use in Military Personnel"

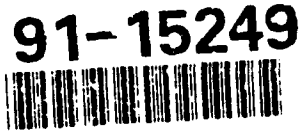
Dear Dr. Melaragno:

This report delineates our accomplishments from March 1, 1991 through July 1, 1991.

Project I: Human Erythropoietin

With our previous triannual report, we submitted a copy of a manuscript. I am please to report that a revised version of this work has been accepted for publication in the European Journal of Biochemistry. This journal is a very prestigious journal known worldwide. Because of this wide readership, this work supported by the U.S. Navy Grant will be rapidly disseminated in both the United States and abroad. Additionally, since our previous report, we submitted a short manuscript which was accepted and published in Biochemical and Biophysical Research Communications. We have enclosed a copy of the manuscript accepted by the European Journal of Biochemistry and a reprint from Biochemical and Biophysical Research Communications.

We have continued our work on the new series of erythropoietin mutants. Interestingly, in sequencing the first five mutants that we derived, we discovered that another mutation had occurred spontaneously. In the short term, this has resulted in some delay in our studies, since we now feel it necessary to sequence not only the mutated domain of each cDNA, but rather the



entire cDNA to verify the absence of other spontaneous mutations. However, in the long term, it is to our benefit to realize that the state-of-the-art methodology that we have employed in preparing these mutants is subject to some technical artifacts. Discovering this at this early stage of our studies will save us many problems in the long run. We expect that within the next three to six months, we will have finished all of our sequencing and will begin to express some of these mutants and initiate their biological characterization.

We completed our first round of studies on developing erythropoietin-erythropoietin dimers. Using the chemical modifying reagents SPDP and SMCC, we derived a series of erythropoietin dimers, trimers and tetramers. Unfortunately, biological studies reveal that these higher ordered erythropoietins were devoid of significant biological activity. There are two possible reasons for this. Firstly, the degree of derivatization by SMCC or SPDP may have been too great, resulting in inactivation of critical amino acid residues. Secondly, the covalent attachment of biologically active erythropoietin molecules may have resulted in the introduction of sufficient steric hindrance to render the molecules inactive. In the next three months, we will explore the use of analogs of SPDP and SMCC which contain "spacer arms", so called "long chain" SPDP. These spacer arms will result in dimers that are separated by 10 to 16 Angstroms, thus increasing steric freedom. Analysis of such erythropoietin dimers will permit us to determine whether inactivation by excess modification or steric factors were to blame for our initial difficulties. We also are exploring the use of polyethylene glycol (PEG) as a means to derivatize erythropoietin and increase its biological half-life.

Project II: Erythroid Burst Promoting Activity

Our studies of BPA indicate that it is a pleotropic erythroid specific growth factor active on both early and late erythroid progenitors. Because of this, we have designated this molecule erythroid colony stimulating factor (E-CSF) in order to distinguish it from other growth factors with "BPA-like" activity. In the next period, we will focus on deriving a rapid and sensitive biological assay for E-CSF employing [^{3}H]thymidine uptake into target erythroid cells. The importance of achieving such an assay is great. It will permit the purification process and analysis of E-CSF containing solutions to be greatly expedited.

Summary

Progress on both Project I and Project II continues. We have encountered some unforeseen methodological problems in both projects. However, in the last project period we have succeeded in publishing one paper and having a second paper accepted for publication.

Truly yours,

Arthur J. Sytkowski, M.D.
Director, Laboratory for Cell
and Molecular Biology

AJS:rck

enclosures

cc: Dr. Laurie Feldman
Research Administration
George Kilbride, Administrative Grants Officer
Defense Technical Information Center